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Research Article

Application of bacterial and yeast biosurfactants for enhanced removal and biodegradation of motor oil from contaminated sand

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ABSTRACT

Background: This study investigated the potential application of two biosurfactants for enhanced removal capability and biodegradation of motor oil contaminated sand under laboratory conditions. The biosurfactants were produced by the yeast *Candida sphaerica* and by the bacterium *Bacillus* sp. cultivated in low-cost substrates. The ability of removing motor oil from soil by the two biosurfactants was identified and compared with that of the synthetic surfactants Tween 80 and Triton X-100.

Results: Both crude and isolated biosurfactants showed excellent effectiveness on motor oil removal from contaminated sand under kinetic conditions (70–90%), while the synthetic surfactants removed between 55 and 80% of the oil. A contact time of 5–10 min under agitation seemed to be enough for oil removal with the biosurfactants and synthetic surfactants tested. The crude and the isolated biosurfactant from *C. sphaerica* were able to remove high percentages of motor oil from packed columns (around 90%) when compared to the biosurfactant from *Bacillus* sp. (40%). For the degradation experiments conducted in motor oil contaminated sand enriched with sugar cane molasses, however, oil degradation reached almost 100% after 90 d in the presence of *Bacillus* sp. cells, while the percentage of oil degradation did not exceed 50% in the presence of *C. sphaerica*. The presence of the biosurfactants increased the degradation rate in 10–20%, especially during the first 45 d, indicating that biosurfactants acted as efficient enhancers for hydrocarbon biodegradation.

Conclusions: The results indicated the biosurfactants enhancing capability on both removal and rate of motor oil biodegradation in soil systems.

1. Introduction

In recent years, much attention has been directed towards biosurfactants owing to their different advantages such as lower toxicity, higher biodegradability, better environmental capability, higher foaming, high selectivity, specific activity at extreme temperatures, pH and salinity, and the ability to be synthesized from renewable feed stocks [1]. Some disadvantages can be mentioned for the use of biosurfactants: at the time, a small amount of biomolecules is produced at industrial level. Many biosurfactants are yet in a laboratory scale level and some of them are quite expensive. The discovery of new biosurfactants, development of new fermentation and recovery processes and the use of cheap raw materials (specifically the use of agro-industry wastes as carbon sources) will allow that more inexpensive biosurfactants can be available for remediation process [2].

The major difficulty in bioremediation of oil-contaminated soil is the bioavailability or mass transfer limitation of the oil pollutants in the soil, causing poor food-microorganism contact and thus poor biodegradation efficiency [3]. Oil penetration through soil is an extremely complex process related to physical, chemical, and biological factors [4]. Petroleum hydrocarbons are highly hydrophobic material with low water solubility and those components attach to soil particles, reducing the bioavailability of oil compounds to microorganisms, thereby limiting the rate of mass transfer for biodegradation. The possible physical forms for oil contaminants in soil can be dissolved in pore water, adsorbed onto soil particles, absorbed into soil particles, or be present as a separate phase, which can be a liquid or a solid phase [3]. The key process to enhance the bioavailability of the oil contaminant is to transport the pollutant to the aqueous bulk phase [5]. One of the effective ways to increase the bioavailability (or solubility) of hydrophobic pollutants in soil is using surfactants to enhance the desorption and solubilization of petroleum

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hydrocarbons, thereby facilitating their assimilation by microorganisms [5,6,7].

Enhanced soil washing generally has been performed with synthetic surfactants, including anionic, nonionic, cationic and mixed surfactants, and some of them have shown great washing capabilities for hydrophobic organic compounds (HOCs) from contaminated soils and groundwater [8]. Some synthetic surfactants, such as Triton X-100, Tween 80, Afonic 1412-7, are shown to be able to enhance the concentration of nonpolar compounds in the aqueous phase [5,6]. However, the residual synthetic surfactants in soils and groundwater have the potential toxicity risk or hazard to environment and human health. So, an improved strategy for soil washing technology is to use biosurfactants [9]. Therefore, biosurfactants seem to be better candidates for using in soil washing technology. The literature data indicated that most of previous studies have focused on few biosurfactants [5,10,11]. More other biosurfactants should be investigated for their properties in enhancing soil washing because they may have more promising properties [9].

At low concentrations, biosurfactants are soluble in water, and with increasing concentrations, they form micelle in solution. The concentration at which micelle begins to form is called the critical micelle concentration (CMC); above the CMC, biosurfactants can solubilize petroleum hydrocarbons in soil-water systems, but some biosurfactants may increase the water solubility of hydrocarbon molecules below the CMC. Therefore, biosurfactants may be useful in degradation of soil contaminating hydrocarbons [12].

The aims of this work were to use two biosurfactants, *i.e.*, a glycolipid produced by *Candida sphaerica* [13] and another new biosurfactant produced by *Bacillus* sp. to remove motor oil from a laboratory oil-contaminated sand and to compare their efficiency with two commonly used synthetic surfactants (Tween 80, and Triton X-100) in agitated (flasks) and static assays (packed columns). Additionally, potential application of the two biosurfactants for enhanced biodegradation of motor oil contaminated sand with a series of bench-scale experiments was evaluated.

2. Materials and methods

2.1. Materials

All chemicals were of reagent grade. Growth media were purchased from Difco Laboratories (USA).

Three types of industrial waste were used as substrates to produce the biosurfactants. Ground nut oil refinery residue was obtained from ASA LTDA in the city of Recife, in Pernambuco state, Brazil. Corn steep liquor was obtained from Corn Products of Brazil in the city of Cabo de Santo Agostinho, Pernambuco, Brazil and sugar cane molasses was obtained from a local plant cane sugar in the city of Igarassu, Pernambuco, Brazil.

Motor oil (15 cSt) was obtained from an automotive maintenance establishment in the city of Recife, Pernambuco, Brazil. We call motor oil to the lubricating oil after use.

2.2. Sand

Samples of 100/50 mesh (0.15–0.3 mm) of Brazilian standard sand NBR 7214 [14] were used in the experiments. Laboratory impregnated sand samples with motor oil were prepared and left to stand at room temperature for 24 h until subsequent use.

2.3. Synthetic surfactants used

Two chemically synthesized surfactants (namely, Tween 80 and Triton X-100) were also used for motor oil removal from contaminated soil to compare their performance with that from biosurfactants. Tween 80 (purchased from Sigma Chemical Co. St. Louis, MO, USA) is a nonionic surfactant and an oil-in-water emulsifier. The CMC of Tween 80 is about 0.0124% (w/v) (120 mg/L) and the surface tension is able to be reduced to 43.7 mN/m. Triton X-100, also obtained from Sigma Chemical Co. (St. Louis, MO, USA), is a nonionic surfactant possessing a hydrophilic polyethylene oxide group and a hydrocarbon lipophilic or hydrophobic group. The CMC of Triton X-100 is about 0.0183% (w/v) (183 mg/L) and the surface tension is able to be reduced to 32.7 mN/m.

2.4. Microorganisms and preparation of seed cultures

C. sphaerica UCP 0995 was obtained from the culture collection of the Universidade Católica de Pernambuco, Brazil. The microorganism was maintained at 5°C on yeast mold agar slants. The *C. sphaerica* inoculum was prepared by transferring cells grown on a slant to 50 mL of yeast mold broth. The seed culture was incubated at 28°C and 150 rpm for 24 h.

The *Bacillus* sp., an indigenous bacterium, was isolated from a petroleum contaminated soil site located in Recife city, Brazil. The bacterium culture was maintained on nutrient agar slants at 4°C. For pre-culture, the strain from a 24 h culture on nutrient agar was transferred to 50 mL of nutrient broth to prepare the seed culture. The cultivation conditions for the seed culture were 28°C, 150 rpm and 10 to 14 h of incubation.

2.5. Production of biosurfactant

The microorganisms were cultivated in a submerged culture in a Marconi MA832 shaker (Marconi LTDA, Brazil).

The yeast biosurfactant was produced in a medium composed of 9% ground nut oil refinery residue and 9% corn steep liquor dissolved in distilled water. The final pH of the medium was 5.3 and the surface tension prior to inoculation was 50 mN/m. The inoculum (1%, v/v) was added to the cooled medium at the amount of 10^4 cells/mL. Fermentation was carried out in 500 mL Erlenmeyer flasks at 28°C and 150 rpm for 144 h [13].

The bacterium biosurfactant was produced in Bushnell-Hass medium (Difco) composed by 0.1% of KH_2PO_4, 0.1% of K_2HPO_4, 0.02% of $MgSO_4 \cdot 7H_2O$, 0.02% of $CaCl_2 \cdot H_2O$ and 0.005% of $FeCl_3 \cdot 6H_2O$. The pH was adjusted to 7.0 by 1.0 M of HCl. The surface tension prior to inoculation was 56 mN/m. Three percent sugar cane molasses and 3% corn steep liquor were added. Two percent aliquots (v/v) of the cell suspension (0.7 optical density at 600 nm), corresponding to an inoculum of 10^7 CFU/mL, were used to inoculate 500 mL Erlenmeyer flasks containing 100 mL of sterile production medium. Cultivation was carried out at 27°C with agitation at 200 rpm for 120 h.

2.6. Determination of surface tension

The CMC of *C. sphaerica* biosurfactant is about 0.025% (w/v) (250 mg/L) and the surface tension is about 25.0 mN/m [13] while the CMC of *Bacillus* sp. biosurfactant was determined as 0.5% (w/v) (5000 mg/L) and the surface tension as 29 mN/m (data not shown).

Since the biosurfactant from *C. sphaerica* was previously produced, measurements of the surface tension were conducted to assess the quality of the biosurfactant obtained. Changes in surface tension were determined in the cell-free broth obtained by centrifuging the cultures at 5000 × g for 30 min. Surface tension was determined using a Sigma 700 Tensiometer (KSV Instruments LTD, Finland) at room temperature. Tensiometers determine the surface tension with the aid of an optimally wettable ring suspended from a precision scale. With the ring method, the liquid is raised until contact with the surface is registered. The sample is then lowered again so that the film produced beneath the liquid is stretched for the determination of maximum force, which is used to calculate the surface tension. The instrument was calibrated against Mill-Q-4 ultrapure distilled water (Millipore, Illinois, USA). Prior to use, the platinum plate and all glassware were

sequentially washed with chromic acid, deionized water and acetone and flamed with a Bunsen burner. Samples were read three times for accuracy.

2.7. Isolation of biosurfactants

The two biosurfactants were extracted from the culture media after cell removal by centrifugation at 5000 × g for 30 min.

The cell-free culture broth from *C. sphaerica* was acidified with 6 M HCl to pH 2.0 and precipitated with two volumes of methanol. After 24 h at 4°C, samples were centrifuged at 5000 × g for 30 min, washed twice with cold methanol and dried at 37°C for 24–48 h [13].

The cell-free culture broth from *Bacillus* sp. had the pH adjusted to 2.0 with 6.0 M of HCl and an equal volume of $CHCl_3/CH_3OH$ (2:1 v/v) was added. The mixture was vigorously shaken for 15 min and allowed to set until phase separation. The organic phase was removed and the operation was repeated twice. The product was concentrated from the pooled organic phases using a rotary evaporator. The viscous yellowish product obtained was dissolved in methanol and concentrated again by evaporation of the solvent at 45°C [15].

2.8. Application of chemical surfactants and biosurfactants in removal of motor oil from sand through kinetic assay

The removal of motor oil from the laboratory contaminated sand was tested through the saturation of 50 g of the standard sand with 10% of motor oil as described by Luna et al. [16]. The laboratory-contaminated soil was placed in 500 mL Erlenmeyer flasks, to which 100 mL of the crude biosurfactants (cell-free broth after fermentation) and isolated biosurfactants and chemical surfactants at 1/2 the CMC, the full CMC and twice the CMC were added. The flasks were shaken at 150 rpm for 5, 10 and 20 min during 24 h at 28°C. The entire content was then centrifuged at 5000 rpm for 1200 s. Control assays were performed using distilled water at the same conditions. The amount of oil residing in the sand after the impact of biosurfactant was gravimetrically determined as the amount of material after extraction with hexane and the % of oil removal was calculated using the equation:

$$\text{Motor oil removed}\,(\%) = (\text{Oi}-\text{Or})/\text{Oi} \times 100\% \qquad \text{[Equation 1]}$$

where Oi is the initial motor oil in the soil (g) before washing and Or is the motor oil remaining in the soil (g) after washing.

2.9. Application of chemical surfactants and biosurfactants in removal of motor oil from sand packed column through static assay

Glass columns measuring 55 cm in height × 6 cm in diameter were initially filled with approximately 200 g of a mixture containing the sand and 10% of motor oil. The surface was then inundated with 200 mL of the crude biosurfactants (cell-free broth after fermentation) and isolated biosurfactants and chemical surfactants at 1/2 the CMC, the full CMC and twice the CMC under the action of gravity. Percolation of the biosurfactant solution was monitored for 24 h, when no further percolation of the solution was observed [17]. Following the washing of the columns, the soil samples were washed with 20 mL of hexane for the removal of residual oil. The solvent was rotoevaporated at 50°C and the amount of oil removed was determined by gravimetry as described in Section 2.8 [18,19].

2.10. Evaluation of oil-degrading ability in sand

Samples of laboratory contaminated standard sand (10 g) were added to 100 mL of distilled water and the mixture was enriched with 1 mL of sugar cane molasses. Then, solutions of the isolated biosurfactants at their CMC and/or 15% of its microbial-producing species previously cultivated in yeast mold broth and/or nutrient broth (15% inoculum at the amount of 10^8 cells/mL for the yeast and 15% inoculum of 10^7 CFU/mL from a 0.7 optical density at 600 nm for the bacterium) were added and the medium was placed in a rotary shaker at 150 rpm and 28°C for 90 d (Table 1). Experiments were carried out in 250 mL Erlenmeyer flasks. At 15 d of experiment 1% molasses were added to the mixture, totaling five feeds (after 15, 30, 45, 60 and 75 d). Samples of 5 mL were collected every 15 d for hydrocarbons analysis, totaling 6 samples. The percentage of degradation of hydrocarbons was calculated as the concentration of hydrocarbon oil removed from a control prepared without the addition of microorganisms and biosurfactants and analyzed at time 0 [20].

2.11. Total motor oil biodegradation rate

The samples were drawn for estimation of motor oil degradation by gravimetric analysis. The residual motor oil was extracted in a preweighed beaker with hexane in a separating funnel. Extraction was repeated twice to ensure complete extraction. After extraction, hexane was evaporated in a hot air oven at 68–70°C, the beaker was cooled down and weighed.

The % degradation was calculated as follows:

$$\text{Motor oil degradation}\,(\%) = (\text{Od}-\text{Os})/\text{Od} \times 100\% \qquad \text{[Equation 2]}$$

where Od is the amount of motor oil degraded (g) and Os is the amount of motor oil added in the sand (g).

2.12. Statistical analysis

The analyses were performed in triplicates. The mean values and standard deviation (mean ± SD) were calculated and tested. Statistical analysis of variance (ANOVA) was performed on all values and tested for $p < 0.05$ for significance.

3. Results and discussion

3.1. Application of chemical surfactants and biosurfactants in removal of motor oil from sand through kinetic assay

Over decades, chemically synthesized surfactants have been used for enhanced oil recovery (EOR) and for oil spill clean-up. However, because of their toxicity and resistance to degradation, biosurfactants have been studied for a possible replacement of chemical surfactants [10,21].

3.1.1. Effect of biosurfactant concentration on motor oil removal efficiency

Fig. 1 and Fig. 2 displays the results of the experiments carried out in beakers for the removal of motor oil adsorbed to sand by the two biosurfactants.

Biosurfactant concentration is usually a critical factor for the removal of oil compounds from soil. To evaluate the performance of the two biosurfactants in removing motor oil from the contaminated soil, three biosurfactant concentrations were applied to wash the samples

Table 1
Formulated mixtures for motor oil biodegradation experiments in sand.

Experiment	Composition
Set 1	Contaminated sand + sugar cane molasses + *C. sphaerica*
Set 2	Contaminated sand + sugar cane molasses + *Bacillus* sp.
Set 3	Contaminated sand + sugar cane molasses + *C. sphaerica* + *Bacillus* sp.
Set 4	Contaminated sand + sugar cane molasses + *C. sphaerica* biosurfactant + *C. sphaerica*
Set 5	Contaminated sand + sugar cane molasses + *Bacillus* sp. biosurfactant + *Bacillus* sp.
Control	Contaminated sand + sugar cane molasses

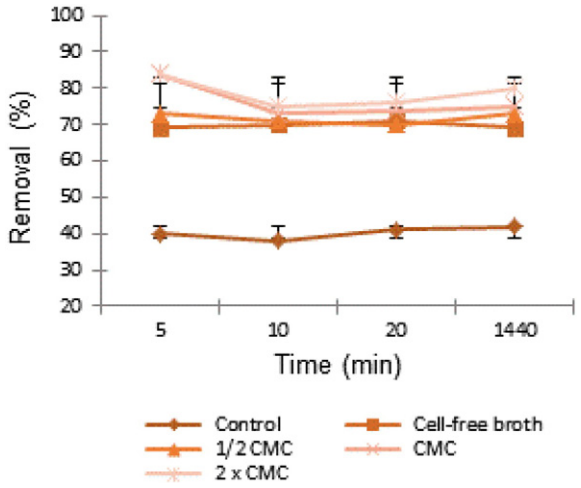

Fig. 1. Removal of motor oil adsorbed to sand through kinetic assay by the biosurfactant from *C. sphaerica*. Error bars show the corresponding standard error.

of sand *i.e.*, under, at and above the CMC. High percentage removals of oil were observed for all solutions tested. The motor oil removal efficiency did not increase with an increase in both biosurfactants concentration. This finding is satisfactory from the environmental stand point, as high concentrations of some biosurfactants have a toxic effect on the native microbial population in the soil [6]. The biosurfactant from *Bacillus* sp. was able to remove a little more oil than the biosurfactant from *C. sphaerica*. Both biosurfactants showed excellent effectiveness on motor oil removal from contaminated sand, thereby being suitable for future application for biostimulation of oil bioremediation in soil. Biosurfactants such as aescin, lecithin, and tannin could not enhance the solubilization of crude oil in soil at concentrations greater than their CMC values [22]. However, when rhamnolipids were used, the solubility of crude oil seemed to increase with an increase in rhamnolipid concentration [5].

Liu et al. [23] showed that the increase in the apparent solubility of some polycyclic aromatic hydrocarbons (PAHs) in the presence of anionic and non-ionic surfactants increases significantly beyond the CMC. Lai et al. [5] evaluated the performance of rhamnolipids and surfactin in removing hydrocarbons from soil, showing that the removal efficiency was positively correlated with the concentration of rhamnolipids and surfactin. The maximum oil removal efficiency of rhamnolipid and surfactin both occurred at 0.2% mass giving a

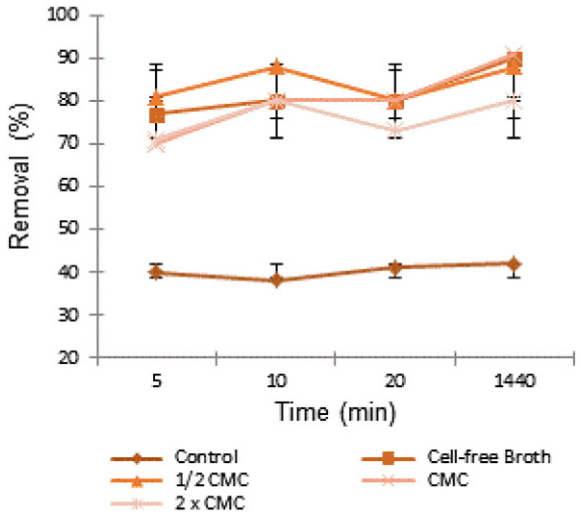

Fig. 2. Removal of motor oil adsorbed to sand through kinetic assay by the biosurfactant from *Bacillus* sp. Error bars show the corresponding standard error.

removal percentage of 23.4 and 14.0, respectively, while the isolated biosurfactants tested in our work removed around 70–80% of the oil. The biosurfactant produced by *Rhodococcus erythropolis* grown on glycerol removed 94% oil in shake flasks [24]. The Rufisan biosurfactant from *Candida lipolytica* at the CMC removed 98% of the oil from beakers in the kinetic assays and biosurfactant concentration exerted no influence on the oil removal rate [19]. On the other hand, the biosurfactant from *C. sphaerica* at 0.1% solution removed 65% of motor oil adsorbed to soil, while the surfactant solution at the CMC (0.08%) removed 55% of the oil and the solution at 0.05% removed approximately 30%, showing the influence of the biosurfactant concentration on the removal rates [25].

As described by Costa et al. [26], two mechanisms are associated with the removal of oil in soils: mobilization and solubilization. Mobilization occurs at concentrations below the CMC and the phenomena associated with this mechanism include the reduction of surface and interfacial tension. Surfactants in contact with the soil/oil system increase the contact angle and reduce the capillary force holding oil and soil together due to the reduction of the interfacial force. Solubilization occurs above the surfactants CMC, as the apparent solubility of oil increases dramatically due to its aggregation within the surfactants micelles. Inside the micelles, the hydrophobic end of the surfactants molecules cluster together forming a hydrophobic environment capable to solubilize hydrophobic substances, while the hydrophilic end exposed to the aqueous phase on the exterior allow the whole structure to remain in solution.

The data observed in this work suggest that mobilization is the main mechanism associated with the removal of motor oil with the biosurfactants and the chemical surfactants, because the increase in (bio) surfactants concentration did not enhance the removal of oil. Besides biodegradability, the removal of oil contaminants without modifying the chemical nature of soil by mobilization is another advantage of biological surfactants over chemical surfactants, as stated by Lai et al. [5].

The biosurfactant from *Pseudomonas aeruginosa* UCP0992 also utilized the mechanism of mobilization to release the oils droplets from sand since the increase of the concentration did not improve the removal of the pollutants [27]. On the other hand, solubilization was the main mechanism associated with the removal of crude oil with the rhamnolipid surfactants produced by *P. aeruginosa* L2-1 from cassava wastewater added with waste cooking oil, because increasing rhamnolipid concentration enhanced the removal of crude oil, due the incorporation of these molecules into micelles [26].

In order to evaluate the use of the crude biosurfactants, the removal ability of the cell-free broth was also tested. The cell-free broth containing biosurfactants and the isolated biosurfactants are almost equally effective in the removal of the motor oil pollutant. Thus, cell-free broth containing biosurfactants can be directly used without purification steps, which would further reduce 30%–50% of the production cost of biosurfactants.

Silva et al. [27] also observed that the cell-free broth containing the crude biosurfactant from *P. aeruginosa* was practically as effective as the isolated biosurfactant when removing 85% diesel oil from sand, thus indicating the possible use of the biosurfactant without purification steps. The cell-free broth from *Candida tropicalis* cultivated in waste frying oil removed approximately 78 to 97% of the petroleum and motor oil adsorbed in sand samples [28]. Over 50% of the oil was extracted after rinsing of the sand with solutions of biosurfactants from *Candida antarctica* [29], while the crude biosurfactant from *Candida guilliermondii* grown in industrial residues removed approximately 90% of the motor oil adsorbed in sand samples [30]. The crude biosurfactant from *C. lipolytica* cultivated in medium containing animal fat and corn steep liquor was more effective in removing motor oil than the isolated biosurfactant [31]. The removal capacity can be affected also by the kind of soil as observed by Silva et al. [15] since the cell-free broth from *Pseudomonas cepacia* grown in

mineral medium supplemented with corn steep liquor and soybean waste frying oil achieved poorer than expected results regarding the removal of motor oil adsorbed to sand, whereas satisfactory results were achieved with clay soil, with removal rates surpassing 80%.

The samples prepared with distilled water (control) showed an interesting result, since it was possible to remove around 40% of the oil adsorbed in the sand. Our results are in accordance with the literature since Chang et al. [32] found that 73.6 up to 100% of PAHs were removed in the presence of sodium dodecyl sulfate (SDS), while 30–80% when using only water. According to Khalladi et al. [33], water washing of a diesel-polluted soil could eliminate up to 24% of *n*-alkanes. This low percentage is of great economic interest, especially for important quantity of polluted soil. Therefore, a water washing process can be recommended before any other remediation process to reduce the hydrocarbon soil content and subsequently the consumed surfactant quantity.

The Pseudomonas sp. 2B biosurfactant solution at 0.01% and 0.05% concentrations was able to remove 89% and 92% of the oil adsorbed in the sand, respectively, while the distilled water (control) and synthetic surfactant SDS removed 48% and 63% of the contaminated oil, respectively, while 81% of crude residual oil was removed using the cell-free broth containing the biosurfactant. Similar results were obtained by Abu-Ruwaida et al. [34] for the cell-free broth containing a biosurfactant produced by Rhodococcus cells; 86% of crude residual oil adsorbed in the sand was removed.

3.1.2. Effect of contact time on motor oil removal efficiency

The contact time is also an important parameter affecting the efficiency of oil removal, as a sufficient contact time is required for effective oil removal. In this study, we investigated the effectiveness of oil removal at 5, 10, 20 and 1440 min. As indicated in Fig. 1 and Fig. 2, irrespective of the biosurfactant type and biosurfactant concentration, an increase in contact time from 5 to 1440 min in general led to either a similar motor oil removal efficiency or a slightly decrease in oil removal performance. These results indicate that a contact time of 5–10 min under agitation seemed to be enough for oil removal with the biosurfactants applied. Lai et al. [5] tested the removal efficiency of rhamnolipids and surfactin during 7 d, showing that 1 d was sufficient for solubilization of the hydrocarbons to the mobile phase.

3.1.3. Comparison of motor oil removal efficiency between biosurfactants and synthetic surfactants

For practical application of biosurfactants on oil removal from sand, it is of great interest to compare the performance of biosurfactants with that of two commonly used chemical surfactants (*i.e.*, Tween 80 and TritonX-100). After adding different concentrations of surfactants for 1440 min, it was observed that the contact time of 5–10 min under agitation was also enough for oil removal with the chemical surfactants (Fig. 3 and Fig. 4). It could be observed that the biosurfactants were more effective than the commercially available surfactants. The results indicated the superior performance of 10% of the biosurfactants over chemical surfactants in terms of mobilization of oil pollutants from the contaminated soil and thus the two biosurfactants examined in this work have the potential to be used as biostimulation agents for bioremediation of oil-polluted soils.

Our results are consistent with the results obtained by Lai et al. [5] for two biosurfactants compared to the same chemical surfactants used in this work. The biosurfactant from *Klebsiella* sp. strain RJ-03 grown in sucrose removed about 90% of oil compared to 57–67% recovery by chemical surfactants in shake flasks [35]. Three biosurfactants from *Bacillus subtilis* strains isolated from Brazilian crude oils at a concentration of 1 g/L recovered between 19% and 22% of oil, whereas the recoveries obtained with the chemical surfactants at the same concentration were between 9% and 12% [36]. Another study also investigated the enhanced soil washing of the plant derived natural biosurfactant of *Sapindus* saponin for phenanthrene

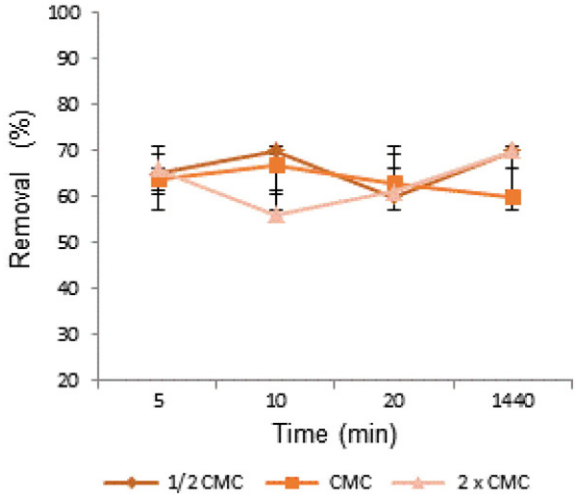

Fig. 3. Removal of motor oil adsorbed to sand through kinetic assay by the synthetic surfactant TritonX-100. Error bars show the corresponding standard error.

from contaminated soil. *Sapindus* saponin could effectively remove phenanthrene from contaminated soil with a maximum removal percentage of about 87.4%, which was only slightly less than that of Tween 80 [9]. Liu et al. [37] showed that surfactin and the chemical surfactant SDS and polyethylene glycol monododecyl ether (PGME) could remove more than 95% of artificial crude oil from sand.

3.2. Application of chemical surfactants and biosurfactants in removal of motor oil from sand packed column through static assay

Laboratory studies on MEOR typically use sand-packed columns, which provide a suitable bench-scale approach to evaluate oil recovery for several reasons: it is an economic model; a battery of columns can be set up simultaneously; and they can simulate the oil recovery operations usually conducted in reservoirs [38].

In this work, a sand-packed column was used to study the effect of two biosurfactants and two chemical surfactants on solubilization of entrapped oil.

The crude and the isolated biosurfactant produced by *C. sphaerica* were able to remove high percentages of motor oil from packed columns when compared to the biosurfactant from *Bacillus* sp. (Table 2). Based on its high surface activity, the biosurfactant from *C. sphaerica* seems to have the potential for the use in mobilizing crude oil in biostimulation processes. It was also observed that the use of the

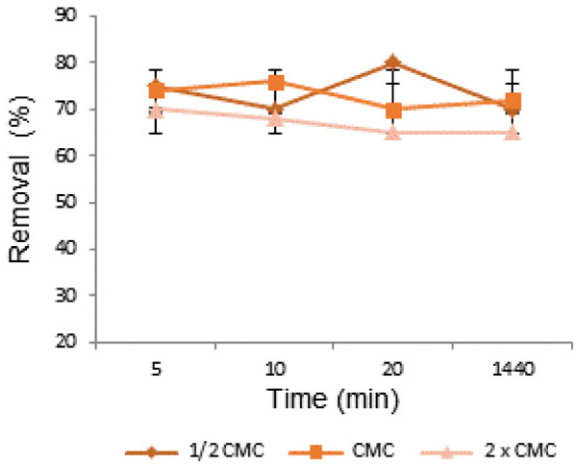

Fig. 4. Removal of motor oil adsorbed to sand through kinetic assay by the synthetic surfactant Tween 80. Error bars show the corresponding standard error.

crude biosurfactant is sufficient to reach the best removal percentages values and that the biosurfactant concentrations did not influence the removal rates of motor oil. Studies carried out by Urum et al. [22] demonstrated that the mobilization or solubilization of hydrophobic compounds by surfactants in sand packed columns may or may not vary depending on the concentration employed. Some surfactants of a vegetal origin, such as aescin, lecithin and tannin, were not capable of enhancing the solubilization of hydrophobic compounds at concentrations above the CMC.

Crude biosurfactants from *P. aeruginosa* isolates cultivated in glycerol removed 49–54% of crude oil contained in packed columns [12]. High concentrations (2.5 and 5.0 g/L) of a biosurfactant isolated from *P. aeruginosa* 57SJ (CMC 400 mg/L) were needed to remove 70% of pyrene adsorbed to soil [39].

The removal of motor oil in packed glass columns by the biosurfactant from *C. lipolytica*, on the other hand, showed the influence of biosurfactant concentration since removal rates of the percolating liquids obeyed the following increasing order: distilled water (7%), Tween 80 (12%), cell-free broth (26%), biosurfactant at the CMC (33%) and biosurfactant at three times the CMC (37%) [19].

The biosurfactants produced by *Bacillus* species cultivated in residues of molasses and cheese whey removed about 30% of the oil contained in a packed column [20]. The oil removal activity of surfactin had been evaluated by sand packed test with fresh kerosene contaminated soil, showing a 34–62% oil recovery by flushing with 0.1 mass % surfactin solution [40,41]. Cameotra and Makkar [41] had demonstrated that the biosurfactant isolated from *P. aeruginosa* was able to recover 56% of the oil adsorbed to the sand contained in a column.

It is interesting to observe that the experiments under static conditions allowed removal percentages similar to the experiments in flasks, showing that the agitation did not increase the interaction between the biosurfactant from *C. sphaerica* and the contaminant. Such behavior was not observed for the *Bacillus* sp. biosurfactant and for the chemical surfactants since the kinetic experiments allowed better removal rates compared to sand packed columns. Lee et al. [42] obtained a removal ratio of 73 and 95% in batch and column experiments, respectively.

The performance of water in the removal of motor oil was negligible as shown in Table 2. Khalladi et al. [33], on the other hand, showed that the performance of water in the removal of diesel fuel was found to be non-negligible, while water contributed by 24.7% in the global elimination of *n*-alkanes. The biosurfactant produced by a crude oil degrading bacteria was tested for oil recovery in sand packed column showing an oil recovery efficiency of 76% compared to the control in which only 30% of the oil was recovered over the same period [43].

According to Zhou et al. [9] sorption of surfactants onto soil would decrease the effective concentrations of surfactant in aqueous solution to solubilize HOCs, and the soil-sorbed surfactants can also enhance soil retardation capability for HOCs, both of which would reduce soil washing efficiency and result in an increase in remediation time and cost. The results obtained in this work suggest that the two biosurfactants studied did not show a strong interaction with the soil.

The chemical surfactant Triton X-100 removed similar quantities of motor oil in both kinetic and static experiments. It is interesting to observe that the removal efficiency was positively correlated with the concentration of Triton X-100 under static conditions while the agitation allowed no difference between the rates of removal under kinetic experiments. Tween 80, on the other hand, removed practically half the oil removed when applied in the sand packed column when compared to the experiments under kinetic conditions. The increase in concentration of the surfactant did not improve the oil removal rates, as observed in the kinetic assays.

In general, biosurfactants exhibit more ability to remove hydrophobic contaminants under static conditions than chemical surfactants, although results may vary depending on the type of surfactant, its concentration and the kind of soil, which can potentiate the interaction with the surfactant more than the interaction between surfactant and oil.

Microbially produced biosurfactants were studied to enhance crude oil desorption and mobilization in model soil column systems. The ability of biosurfactants from *Rhodococcus ruber* to remove the oil from the soil core was 1.4–2.3 times greater than that of a synthetic surfactant of suitable properties, Tween 60. Biosurfactant was less adsorbed to soil components than synthetic surfactant, thus rapidly penetrating through the soil column and effectively removing 65%–82% of crude oil [4].

Sobrinho et al. [44] observed removals around 75% and 92% depending on the soil type with the crude biosurfactant from *C. sphaerica* cultivated in industrial residues, while percentages removal between 30% and 50% were obtained for the isolated biosurfactant in the soils contained in packed columns. The synthetic surfactant Tween 20 and the distilled water removed around 20% of the oil in the soils tested.

The washing process of a soil column by the ionic surfactant SDS was investigated. The effect of SDS was significant beyond a concentration of 8 mM. The soil washing process had removed 97% of the diesel fuel [33].

Like the cell-free broth from *C. sphaerica*, the culture broth from *Rhodococcus* sp. strain TA6 grown on sucrose was effective in recovering up to 70% of the residual oil from oil-saturated sand packed. Comparison of the results (SDS 0%, spolene 63% and petroleum sulfonate 58%) with residual oil recovery obtained by TA6 culture broth indicated the potential value of the biosurfactant for EOR [45].

Jain et al. [35] investigated the potential use of two biosurfactants in removing oil in glass columns compared to synthetic surfactants. The results showed the efficiency of biosurfactants produced by *B. subtilis* PT2 and *P. aeruginosa* SP4 in removing oil. They exhibited values of 68% and 57%, respectively, compared to the synthetic surfactants Tween 80 (52%), SDBS (51%) and Alfoterra 5PO-145 (55%).

Bai et al. [46] investigated the potential of an anionic rhamnolipid isolated from *P. aeruginosa* for the removal of hydrocarbons adsorbed to soil in packed columns. The biosurfactant was able to remove 84% of hexadecane absorbed to sand with particles measuring 0.6–0.85 mm (mesh 20/30), whereas a 22% removal rate was found for sand particles measuring 0.3–0.42 mm (mesh 40/50). The removal capacity of the rhamnolipid using 40/50 mesh was compared with that of two synthetic surfactants: the anionic SDS (CMC 2360 mg/L) and the non-ionic Tween 80 (CMC 13 mg/L). SDS (472 mg/L) and Tween 80 (51 mg/L) removed 0% and 6% of the hexadecane, respectively.

Table 2
Removal of motor oil adsorbed to sand in packed columns (static assay) by the biosurfactants produced by *C. sphaerica* and *Bacillus* sp. and by the chemical surfactants Tween 80 and Triton X-100.

Surfactant types	Removal of motor oil by percolating liquids (%)			
	Crude biosurfactant	(Bio) surfactant (1/2 CMC)	(Bio) surfactant (CMC)	(Bio) surfactant (2 × CMC)
Produced by *C. sphaerica*	93 ± 3.9	87 ± 3.2	92 ± 2.7	91 ± 2.8
Produced by *Bacillus* sp.	43 ± 3.0	15 ± 2.1	30 ± 1.9	40 ± 2.5
Tween 80	–	45 ± 2.1	45 ± 2.0	40 ± 1.8
Triton X-100	–	60 ± 1.0	70 ± 2.1	80 ± 1.5
Distilled water (control)	6 ± 1.0	–	–	–

3.3. Motor oil biodegradation

Five different sets were used to study motor oil biodegradation. The results were recorded on 15, 30, 45, 60, 75 and 90th d for each set as shown in Fig. 5.

The addition of molasses provided required nutrients for enhanced growth of the microorganisms and the biodegradation of the petroleum derivate. Molasses is a co-product of sugar production, both from sugar cane as well as from sugar beet industry in Brazil. Molasses is rich in carbon, organic nitrogen and mineral compounds required for growth of microorganisms. Therefore, molasses was added to the mixtures of contaminated sand along the experiments.

In the first set of experiment (Contaminated sand + sugar cane molasses + *C. sphaerica*), the oil degradation reached 50% after 90 d. The same percentage was obtained in the presence of the biosurfactant (Set 4), which accelerated the oil degradation during the first 75 d. On the other hand, the percentage of degradation in the second Set was much higher, reaching almost 100% in the presence of *Bacillus* sp. cells. The presence of the biosurfactant produced by *Bacillus* sp. also accelerated the degradation process in the first 45 d of the experiment (Set 5), *i.e.*, the biosurfactant increased the degradation rate in 10%, indicating that biosurfactant acted as an efficient enhancer for hydrocarbon biodegradation. It may be due to i) increase in the surface area of hydrophobic water-insoluble substrates and ii) increase in the bioavailability of hydrophobic compounds [47]. The presence of both microorganisms, namely yeast and bacterium used together (Set 3) was not efficient in the degradation of the oil, which did not exceed 50%. As described by Luna et al. [16], the biosurfactant from *C. sphaerica* expressed antimicrobial properties against a variety of microorganisms, suggesting the possible inhibition of the growth of *Bacillus* sp. by the biosurfactant produced by the yeast. Degradation was not observed in the control set of experiment (contaminated sand + sugar cane molasses).

Variable results have been shown concerning the utility of using biosurfactants in hydrocarbon solubilization and biodegradation [39, 48]. According to Zheng et al. [49], the solubilizing capacity of a specific surfactant is determined only by its intrinsic micelles property and thus enhancing its solubilizing capacity is usually very difficult. Therefore, continuing efforts have been made to search for new surfactants or biosurfactants with much higher solubilizing efficacy, lower cost and low microbial toxicity.

Oberbremer et al. [50] used a mixed soil population to assess hydrocarbon degradation in a model oil system. They reported a statistically significant enhancement in hydrocarbon degradation when sophorose lipids were added to the system containing 10% soil and a 1.35% hydrocarbon mixture in the mineral salt medium. In the absence of surfactant, 81% of the hydrocarbon mixture was degraded within 114 h, while in the presence of biosurfactant up to 90% of the hydrocarbon mixture was degraded within 79 h.

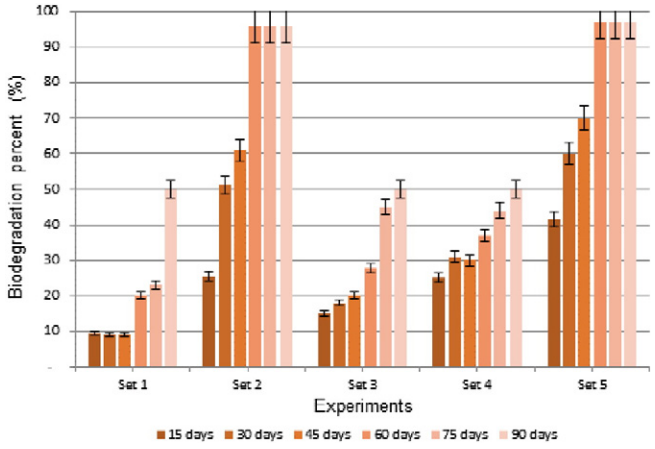

Fig. 5. Biodegradation of motor oil. Set 1 — contaminated sand + sugar cane molasses + *C. sphaerica*; Set 2 — contaminated sand + sugar cane molasses + *Bacillus* sp.; Set 3 — contaminated sand + sugar cane molasses + *C. sphaerica* + *Bacillus* sp.; Set 4 — contaminated sand + sugar cane molasses + *C. sphaerica* biosurfactant + *C. sphaerica*; Set 5 — contaminated sand + sugar cane molasses + *Bacillus* sp. biosurfactant + *Bacillus* sp. Error bars show the corresponding standard error.

The biosurfactant from *Oceanobacillus* sp. BRI 10 was tested in crude oil biodegradation experiments. The percent degradation reached 63% in the first set of experiment (basal salt medium + crude oil + bacterial cells) on the 27th d. On the other hand, it was around 90% in the second set of experiment (basal salt medium + crude oil + bacterial cells + biosurfactant) [47].

Two biosurfactants, surfactin and rhamnolipid, were applied for enhanced biodegradation of diesel contaminated water and soil with a series of bench-scale experiments. The addition of surfactin near its CMC increased diesel biodegradation percentage (94%), compared to batch experiments with no surfactin addition (40% biodegradation percentage). Addition of surfactin more than 40 mg/L, however, decreased diesel biodegradation efficiency. Addition of rhamnolipid to diesel/water systems, from 0 to 80 mg/L (CMC at 50 mg/L), substantially increased diesel biodegradation percentage, from 40 to 100%, respectively. Rhamnolipid addition at a concentration of 160 mg/L provided similar results to those of an 80 mg/L addition [51].

The effects of the addition of the biosurfactant from *P. cepacia* alone and with cells of the bacterium in the biodegradation process of HOCs adsorbed to soil were studied during 60 d. Results indicated the efficiency of both the biosurfactant and its producing species in degrading high percentages of the HOCs adsorbed to the soil samples [52].

Youssef et al. [53] described the injection of a glucose–nitrate–mineral nutrient mixture and two lipopeptide biosurfactant producing Bacillus strains into two wells to correlate *in-situ* metabolism with oil recovery. Analysis of production water indicated *in-situ* growth of the injected strains and other heterotrophic fermenting bacteria, metabolism of the nutrients, and biosurfactant production.

Most studies describe the use of bacteria in the degradation of HOCs although the efficiency of yeast has also been demonstrated. The efficacy of *Candida catenulata* CM1 on petroleum hydrocarbon degradation was evaluated during composting of a mixture containing 23% food waste and 77% diesel contaminated soil including 2% (w/w) diesel. After 13 d of composting, 84% of the initial petroleum hydrocarbon was degraded [54].

4. Conclusions

It could be observed in the present study that the two biosurfactants were more effective than the commercially available surfactants tested. The cell free broth containing biosurfactants and the isolated biosurfactants are almost equally effective in the removal of the oil pollutant. Thus, cell free broth containing biosurfactants can be directly used without purification steps, which would further reduce the cost of production of the biosurfactants. The biosurfactant produced by *C. sphaerica* could be applied in enhanced oil recovery operations, while the biosurfactant produced by *Bacillus* sp. should more suited for enhanced biodegradation of petroleum derivates in soil systems.

Conflict of interest

There is no conflict of interest.

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References

[1] Silva RC, Almeida DG, Rufino RD, Luna JM, Santos VA, Sarubbo LA. Applications of biosurfactants in the petroleum industry and the remediation of oil spills. Int J Mol Sci 2014;15:12523–42. http://dx.doi.org/10.3390/ijms150712523.

[2] Torres L, Moctezuma A, Avendaño JR, Muñoz A, Gracida J. Comparison of bio- and synthetic surfactants for EOR. J Pet Sci Eng 2011;76:6–11. http://dx.doi.org/10.1016/j.petrol.2010.11.022.

[3] Paria S. Surfactant-enhanced remediation of organic contaminated soil and water. Adv Colloid Interface Sci 2008;138:24–58. http://dx.doi.org/10.1016/j.cis.2007.11.001.

[4] Kuyukina MS, Ivshina IB, Makarov SO, Litvinenko LV, Cunningham CJ, Philp JC. Effect of biosurfactants on crude oil desorption and mobilization in a soil system. Environ Int 2005;31:155–61. http://dx.doi.org/10.1016/j.envint.2004.09.009.

[5] Lai CC, Huang YC, Wei YH, Chang JS. Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. J Hazard Mater 2009;167: 609–14. http://dx.doi.org/10.1016/j.jhazmat.2009.01.017.

[6] Christofi N, Ivshina IB. A review: Microbial surfactants and their use in field studies of soil remediation. J Appl Microbiol 2002;93:915–29. http://dx.doi.org/10.1046/j.1365-2672.2002.01774.x.

[7] Mulligan CN, Yong RN, Gibbs BF. Surfactant-enhanced remediation of contaminated soil: A review. Eng Geol 2001;60:371–80. http://dx.doi.org/10.1016/S0013-7952(00)00117-4.

[8] Urum K, Grigson S, Pekdemir T, McMenamy S. A comparison of the efficiency of different surfactants for removal of crude oil from contaminated soils. Chemosphere 2006;62:1403–10. http://dx.doi.org/10.1016/j.chemosphere.2005.05.016.

[9] Zhou W, Wang X, Chen C, Zhu L. Enhanced soil washing of phenanthrene by a plant-derived natural biosurfactant, *Sapindus saponin*. Colloids Surf A Physicochem Eng Asp 2013;425:122–8. http://dx.doi.org/10.1016/j.colsurfa.2013.02.055.

[10] Mulligan CN. Environmental applications for biosurfactants. Environ Pollut 2005; 133:183–98. http://dx.doi.org/10.1016/j.envpol.2004.06.009.

[11] Mulligan CN. Recent advances in the environmental applications of biosurfactants. Curr Opin Colloid Interface Sci 2009;14:372–8. http://dx.doi.org/10.1016/j.cocis.2009.06.005.

[12] Bordoloi NK, Konwar BK. Microbial surfactant-enhanced mineral oil recovery under laboratory conditions. Colloids Surf B Biointerfaces 2008;63:73–82. http://dx.doi.org/10.1016/j.colsurfb.2007.11.006.

[13] Luna JM, Rufino RD, Sarubbo LA, Campos-Takaki GM. Characterisation, surface properties and biological activity of a biosurfactant produced from industrial waste by *Candida sphaerica* UCP0995 for application in the petroleum industry. Colloids Surf B Biointerfaces 2013;102:202–9. http://dx.doi.org/10.1016/j.colsurfb.2012.08.008.

[14] Associacão Brasileira de Normas Técnicas (ABNT). NBR8492: tijolo maciço de solo-cimento: Determinação da resistência à compressão e da absorcão de água, método de ensaio. Rio de Janeiro; 1982.

[15] Silva NM, Luna JM, Rufino RD, Santos VA, Sarubbo LA. Screening of *Pseudomonas* species for biosurfactant production using low-cost substrates. Biocatal Agric Biotechnol 2014;3:132–9. http://dx.doi.org/10.1016/j.bcab.2013.09.005.

[16] Luna JM, Rufino RD, Sarubbo LA, Rodrigues LRM, Teixeira JAC, Campos-Takaki GM. Evaluation antimicrobial and antiadhesive properties of the biosurfactant Lunasan produced by *Candida sphaerica* UCP 0995. Curr Microbiol 2011;62:1527–34. http://dx.doi.org/10.1007/s00284-011-9889-1.

[17] Robert M, Mercade ME, Bosch MP, Parra JL, Espuny MJ, Manresa MA, et al. Effect of the carbon source on biosurfactant production by *Pseudomonas aeruginosa* 44T1. Biotechnol Lett 1989;11:871–4. http://dx.doi.org/10.1007/BF01026843.

[18] Dahrazma B, Mulligan CN. Investigation of the removal of heavy metals from sediments using rhamnolipid in a continuous flow configuration. Chemosphere 2007;69:705–11. http://dx.doi.org/10.1016/j. chemosphere .2007.05.037.

[19] Rufino RD, Luna JM, Marinho PHC, Farias CBB, Ferreira SRM, Sarubbo LA. Removal of petroleum derivative adsorbed to soil by biosurfactant Rufisan produced by *Candida lipolytica*. J Pet Sci Eng 2013;109:117–22. http://dx.doi.org/10.1016/j.petrol.2013.08.014.

[20] Joshi S, Yadav S, Desai AJ. Application of response-surface methodology to evaluate the optimum medium components for the enhanced production of lichenysin by *Bacillus licheniformis* R2. Biochem Eng J 2008;41:122–7. http://dx.doi.org/10.1016/j.bej.2008.04.005.

[21] Kryachko Y, Nathoo S, Lai P, Voordouw J, Prenner EJ, Voordouw G. Prospects for using native and recombinant rhamnolipid producers for microbially enhanced oil recovery. Int Biodeter Biodegr 2013;81:133–40. http://dx.doi.org/10.1016/j.ibiod.2012.09.012.

[22] Urum K, Pekdemir T, Copur M. Optimum conditions for washing of crude oil-contaminated soil with biosurfactant solutions. Trans IchemE Part B 2003;81: 203–9. http://dx.doi.org/10.1205/095758203765639906.

[23] Liu Z, Jacobson AM, Luthy RG. Biodegradation of naphthalene in aqueous nonionic surfactant systems. Appl Environ Microbiol 1995;61:145–51.

[24] Ciapina EM, Melo WC, Santa Anna LM, Santos AS, Freire DM, Pereira Jr N. Biosurfactant production by *Rhodococcus erythropolis* grown on glycerol as sols carbon source. Appl Biochem Biotechnol 2006;131:880–6. http://dx.doi.org/10.1385/ABAB:131:1:880.

[25] Sobrinho HB, Rufino RD, Luna JM, Salgueiro AA, Campos-Takaki GM, Leite LF, et al. Utilization of two agroindustrial by-products for the production of a surfactant by *Candida sphaerica* UCP0995. Process Biochem 2008;43:912–7. http://dx.doi.org/10.1016/j.procbio.2008.04.013.

[26] Costa SG, Nitschke M, Lépine F, Déziel E, Contiero J. Structure, properties and applications of rhamnolipids produced by *Pseudomonas aeruginosa* L2-1 from cassava wastewater. Process Biochem 2010;45:1511–6. http://dx.doi.org/10.1016/j.procbio.2010.05.033.

[27] Silva SN, Farias CB, Rufino RD, Luna JM, Sarubbo LA. Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP 0992. Colloids Surf B Biointerfaces 2010;79:174–83. http://dx.doi.org/10.1016/j.colsurfb.2010.03.050.

[28] Batista RM, Rufino RD, Luna JM, Souza JED, Sarubbo LA. Effect of medium components on the production of a biosurfactant from *Candida tropicalis* applied to the removal of hydrophobic contaminants in soil. Water Environ Res 2010;82: 1–8. http://dx.doi.org/10.2175/106143009X12487095237279.

[29] Adamczak M, Bednarski W. Influence of medium composition and aeration on the synthesis of surfactants produced by *Candida antarctica*. Biotechnol Lett 2000;22: 313–6. http://dx.doi.org/10.1023/A:1005634802997.

[30] Coimbra CD, Rufino RD, Luna JM, Sarubbo LA. Studies of the cell surface properties of *Candida* species and relation with the production of biosurfactants for environmental applications. Curr Microbiol 2009;58:245–51. http://dx.doi.org/10.1007/s00284-008-9315-5.

[31] Santos DKF, Rufino RD, Luna JM, Santos VA, Salgueiro AA, Sarubbo LA. Synthesis and evaluation of biosurfactant produced by *Candida lipolytica* using animal fat and corn steep liquor. J Pet Sci Eng 2013;12:1–32. http://dx.doi.org/10.1016/j.petrol.2013.03.028.

[32] Chang MC, Huang CR, Shu HY. Effects of surfactants on extraction of phenanthrene in spiked sand. Chemosphere 2000;41:1295–300. http://dx.doi.org/10.1016/S0045-6535(99)00527-5.

[33] Khalladi R, Benhabiles O, Bentahar F, Moulai-Mostefa N. Surfactant remediation of diesel fuel polluted soil. J Hazard Mater 2009;164:1179–84. http://dx.doi.org/10.1016/j.jhazmat.2008.09.024.

[34] Abu-Ruwaida AS, Banat IM, Haditirto S, Salem A, Kadri M. Isolation of biosurfactant-producing bacteria-product characterization, and evaluation. Acta Biotechnol 1991; 11:315–24. http://dx.doi.org/10.1002/abio.370110405.

[35] Jain RM, Mody K, Mishra A, Jha B. Physicochemical characterization of biosurfactant and its potential to remove oil from soil and cotton cloth. Carbohydr Polym 2012;89: 1110–6. http://dx.doi.org/10.1016/j.carbpol.2012.03.077.

[36] Pereira JF, Gudiña EJ, Costa R, Vitorino R, Teixeira JA, Coutinho JA, et al. Optimization and characterization of biosurfactant production by *Bacillus subtilis* isolates towards microbial enhanced oil recovery applications. Fuel 2013;111:259–68. http://dx.doi.org/10.1016/j.fuel.2013.04.040.

[37] Liu Q, Lin J, Wang W, Huang H, Li S. Production of surfactin isoforms by *Bacillus subtilis* BS-37 and its applicability to enhanced oil recovery under laboratory conditions. Biochem Eng J 2015;93:31–7. http://dx.doi.org/10.1016/j.bej.2014.08.023.

[38] Suthar H, Hingurao K, Desai A, Nerurkar A. Evaluation of bioemulsifier mediated Microbial Enhanced Oil Recovery using sand packed column. J Microbiol Methods 2008;75:225–30. http://dx.doi.org/10.1016/j.mimet.2008.06.007.

[39] Bordas F, Lafrance P, Villemur R. Conditions for effective removal of pyrene from an artificially contaminated soil using *Pseudomonas aeruginosa* 57SJ rhamnolipids. Environ Pollut 2007;138:69–76. http://dx.doi.org/10.1016/j.envpol.2005.02.017.

[40] Makkar RS, Cameotra SS. Biosurfactant production by a thermophillic *Bacillus subtilis* strain. J Ind Microbiol Biotechnol 1997;18:37–42. http://dx.doi.org/10.1038/sj.jim.2900349.

[41] Makkar RS, Cameotra SS. Production of biosurfactant at mesophilic and thermophilic conditions by a strain of *Bacillus subtilis*. J Ind Microbiol Biotechnol 1998;20:48–52. http://dx.doi.org/10.1038/sj.jim.2900474.

[42] Lee DH, Cody RD, Kim DJ, Choi S. Effect of soil texture on surfactant-based hydrophobic organic remediation of contaminated soil. Environ Int 2002;27:681–8. http://dx.doi.org/10.1016/S0160-4120(01)00130-1.

[43] Ibrahim ML, Ijah UJ, Manga SB, Bilbis LS, Umar S. Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. Int Biodeterior Biodegrad 2013;81:28–34. http://dx.doi.org/10.1016/j.ibiod.2012.11.012.

[44] Sobrinho HB, Luna JM, Rufino RD, Porto AL, Sarubbo LA. Application of biosurfactant from *Candida sphaerica* UCP 0995 in removal of petroleum derivative from soil and sea water. J Life Sci 2013;7:559–69. http://dx.doi.org/10.17265/1934-7391/2013.06.001.

[45] Shavandi M, Mohebali G, Haddadi A, Shakarami H, Nuhi A. Emulsification potential of a newly isolated biosurfactant-producing bacterium, *Rhodococcus* sp. strain TA6. Colloids Surf B Biointerfaces 2011;82:477–82. http://dx.doi.org/10.1016/j.colsurfb.2010.10.005.

[46] Bai G, Brusseau ML, Miller RM. Biosurfactant-enhanced removal of residual hydrocarbon from soil. J Contam Hydrol 1997;25:157–70. http://dx.doi.org/10.1016/S0169-7722(96)00034-4.

[47] Jadhav VV, Yadav A, Shouche YS, Aphale S, Moghe A, Pillai S, et al. Studies on biosurfactant from *Oceanobacillus* sp. BRI 10 isolated from Antarctic seawater. Desalination 2013;318:64–71. http://dx.doi.org/10.1016/j.desal.2013.03.017.

[48] Chang JS, Radosevich M, Jin Y, Cha DK. Enhancement of phenanthrene solubilization and biodegradation by trehalose lipid biosurfactants. Environ Toxicol Chem 2004; 23:2816–22. http://dx.doi.org/10.1897/03-608.1.

[49] Zheng C, Wang M, Wang Y, Huang Z. Optimization of biosurfactant-mediated oil extraction from oil sludge. Bioresour Technol 2012;110:338–42. http://dx.doi.org/10.1016/j.biortech.2012.01.073.
[50] Oberbremer A, Mühller-Hurtig R, Wagner F. Effect of addition of microbial surfactant on hydrocarbon degradation in soil population in a stirred reactor. Appl Microbiol Biotechnol 1990;32:485–9. http://dx.doi.org/10.1007/BF00903788.
[51] Whang LM, Liu PW, Ma CC, Cheng SS. Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. J Hazard Mater 2008;151:155–63. http://dx.doi.org/10.1016/j.jhazmat.2007.05.063.
[52] Silva EJ, Rocha Silva NM, Rufino RD, Luna JM, Silva RO, Sarubbo LA. Characterization of a biosurfactant produced by *Pseudomonas cepacia* CCT6659 in the presence of industrial wastes and its application in the biodegradation of hydrophobic compounds in soil. Colloids Surf B: Biointerfaces 2014;117:36–41. http://dx.doi.org/10.1016/j.colsurfb.2014.02.012.
[53] Youssef N, Simpson DR, McInerney MJ, Duncan KE. *In-situ* lipopeptide biosurfactant production by *Bacillus* strains correlates with improved oil recovery in two oil wells approaching their economic limit of production. Int Biodeterior Biodegrad 2013;81: 127–32. http://dx.doi.org/10.1016/j.ibiod.2012.05.010.
[54] Joo HS, Ndegwa PM, Shoda M, Phae CG. Bioremediation of oil-contaminated soil using *Candida catenulata* and food waste. Environ Pollut 2008;156:891–6. http://dx.doi.org/10.1016/j.envpol.2008.05.026.